Aging-associated molecular drivers of mitochondrial dysfunction accelerate prostate carcinogenesis

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Background: The majority of prostate cancers are detected in older men, suggesting that the risk of developing prostate cancer increases exponentially with age. Interestingly, oxidative stress and mitochondrial dysfunction, accompanied by the accumulation of mitochondrial DNA (mtDNA) alterations, are also exacerbated with aging. We have previously shown that NKX3.1 expression can stratify patients with dysfunctional mitochondria at higher risk for progression to lethal prostate cancer. We therefore asked whether, and if so, how, NKX3.1 loss cooperates with mtDNA alterations to promote prostate carcinogenesis in the context of aging. Our work aims to identify and characterize novel aging-associated molecular drivers of mitochondrial dysfunction in prostate cancer and assess their prognostic value for high-risk disease.

Methods: We performed preclinical studies using genetically engineered mouse models, human prostate cancer cells and organoids. In particular, to assess the consequences of NKX3.1 loss and mitochondrial dysfunction for prostate cancer progression during aging we developed the *Nkx3.1; PolgA*^{D257A} mutant mice, a new model of accelerated prostate carcinogenesis where a defective mitochondrial DNA polymerase leads to accumulation of mutated mtDNA with age. We employed a systems biology approach to identify mtDNA mutations and master regulators (MRs) in aging-associated prostate cancer and, established mtDNA base-editing tools in human prostate cancer LNCaP cells and patient-derived organoid expressing or lacking NKX3.1 for functional validation studies.

Results: We found that the tumorigenic consequences of NKX3.1 loss are enhanced as aging prostate cells acquire mtDNA mutations. Specifically, our analyses of mouse models having loss of function of Nkx3.1 combined with accumulated mtDNA mutations revealed an aging-associated acceleration of prostate cancer with evident mitochondrial dysfunction and aging hallmarks, including cellular senescence, oxidative stress, and telomere attrition. Targeted molecular analyses identified mtDNA control region mutations uniquely present in the more aggressive prostate tumors of older mice, which together with alterations in NKX3.1 copy numbers, stratified patients with poor prognosis. In addition, introduction of mtDNA mutations in human prostate cancer cells and organoids lacking NKX3.1 expression promoted tumorigenesis. Finally, MR analyses on gene expression profiles from young vs old mouse prostate tissues identified promising candidate MRs that intersect prostatic aging and carcinogenesis, including known molecular drivers of tumor initiation, hypoxia and redox pathways.

Conclusions: Our studies provide fundamental insights into the synergy of NKX3.1 loss with mitochondrial dysfunction for prostate cancer initiation and progression, as well as unique models to unravel their relationship to prostatic aging. Now, leveraging the molecular data from our mouse models and data from human prostate cancer cohorts, we are interrogating regulatory and mitochondrial genome networks to identify conserved MRs for high-risk human prostate cancer. Following prioritization of candidate aging-associated and clinically-relevant MRs, we will perturb our human patient-derived organoids to investigate their impact on prostatic mitochondrial function and tumorigenesis.

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